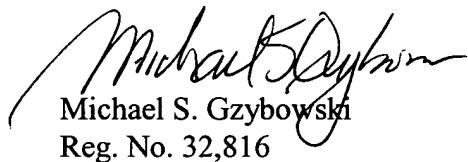


•• R E M A R K S / A R G U M E N T S ••

By the Present Preliminary Amendment corrects matters of grammar, sentence structure, syntax, sentence structure form in the specification and claims without changing the scope of the disclosure or adding any new matter thereto.

Entry of the present Preliminary Amendment prior to the examination of the present application is respectfully requested.

Respectfully submitted,



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132483.1

Asiaticoside-liposome and the use thereof

Technical field

This invention belongs to the chemical field, which field and is related to the fields of pharmaceutical preparations and cosmetic, especially cosmetics. More specifically, the present invention is directed to asiaticoside-liposome asiaticoside-liposomes and its their use for in the preparing preparation of pharmaceutical preparations compositions and cosmetic. cosmetics.

Background technology

Centella asiatica(L.)Urban belongs to Umbelliferae. the Umbellifera family. Its herb can be used as an officinal, which has the effects of defervescence, diuretic, detoxicating and anti-swelling detoxicating, anti-swelling, etc. As a folk medicine in China, the extract of *Centella asiatica* is used as a remedy for jaundice with damp-heat pathogen, wound and dermal ulcer wounds, dermal ulcers, etc. The existing Existing data indicates that the component of triterpene saponins extracted from *Centella asiatica* can distinctly facilitate the wound healing process, stimulate the growth of the granulation, promote the keratinization of the epidermis, and redound to allow the generation of new connective tissue, which tissue. In addition, the component of triterpene saponins extracted from *Centella asiatica* can also be used as a remedy for burn, the burns, lower limb ulcers, wounds, adhesion of tendons, limbs' ulcer, wound and adhesion of tendon etc. Moreover, asiaticoside shows significant activity for scar-hyperplasia and keloid, and it can prevent skin from erythema induced by ultraviolet irradiation. Therefore it becomes a research hotspot that much interest exists for developing asiaticoside would be developed into functional cosmetic to cosmetics that can prevent and cure cutaneous disease. diseases.

Asiaticoside is a triterpene saponin. While practicing use, Attempts at practical use find that asiaticoside is found that it can hardly permeate skin because of its big molecular weight (approximate 936), bad liposolubility and water-solubility; asiaticoside water-solubility. In addition, asiaticoside is instable in air or and solutions and can easy to be oxidized oxidated and degraded because of the character of its structure, which structure. These factors influence the preparing for ability to prepare stable pharmaceutical preparations and cosmetic prescription; cosmetics. Moreover, bad liposolubility and water-solubility result in difficulties with the preparation process that because asiaticoside can not be mixed with other components of pharmaceutical preparations and cosmetic cosmetic compositions and formulations. These disadvantageous factors restrict the further development and the application of asiaticoside in the field of pharmaceutical preparations compositions and formulations that are intended to be administered per cutem and cosmetic cosmetic compositions and formulations. Therefore, a need exists it is very important to find a kind of suitable drug-carrier which can enhance the chemical stability and skin penetrability of asiaticoside so as to be convenient for the preparation of its pharmaceutical preparations and cosmetic.

Disclosure of the Invention

One aspect of this the present invention is to provide a asiaticoside-liposome asiaticoside-liposomes for skin use, in allusion to the shortcoming lies in asiaticoside's to overcome the previous inability to use asiaticoside using in pharmaceutical preparations that are intended to be administered per cutem and cosmetic cosmetics.

Another aspect of this the present invention is to provide for the use of asiaticoside-liposome asiaticoside-liposomes for preparing pharmaceutical preparations compositions and formulations and cosmetic cosmetics which contain asiaticoside.

Invention content

Best Mode for Carrying out the Invention

Asiaticoside-liposome is The asiaticoside-liposomes of the present invention are a kind of opalescent suspension. It is just necessary to uniformly mix asiaticoside-liposome the asiaticoside-liposomes with the other components of prescription uniformly when preparing pharmaceutical preparations compositions and formulations and cosmetics cosmetic. The asiaticoside-liposome asiaticoside-liposomes for skin use is-a-kind-of are hydrophilic opalescent suspensions in which the asiaticoside is enwrapped in the middle of liposome bilayer membranes. This The present invention can enhance not only asiaticoside's stability but also its skin penetrability and hydrophilicity, and it is more propitious to prepare pharmaceutical preparations compositions and formulations and cosmetic cosmetics of asiaticoside. asiaticoside according to the present invention.

The asiaticoside-liposome asiaticoside-liposomes for skin use dislosed by this provided by the present invention is prepared by the following methods and steps:

1. Asiaticoside monomer is isolated from the total saponins of *Centella asiatica* according to conventional methods;
2. The said asiaticoside and lipid components used in the liposomes prescription compositions and formulations are fused by heating or dissolved dissolution in organic solvents to make a lipid solution;
3. The said lipid solution is placed into rotary evaporator, then a lipid film is afforded produced at the bottom of the vessel by the rotary thin layer evaporation technique;
4. Lipid A lipid dispersing aqueous solution is afforded produced after the said lipid film had has been hydrated by adding an aqueous solution under shaking, while shaking the resulting mixture, or afforded by mixing the lipid solution mentioned in from step 2 with an aqueous solution directly under shaking;

5. Asiaticoside-liposome The asiaticoside-liposome is obtained after the said lipid dispersing aqueous solution has been treated by using the techniques techniques of sonication, sonification, homogeneous emulsification, microjet and extruding filtration.

Asiaticoside The asiaticoside content is 0.1~10% in the asiaticoside-liposome asiaticoside-liposomes developed for skin use disclosed by this invention. according to the present invention is 0.1~10%.

In the liposomes prescription compositions and formulation of this the present invention, ceramide is included in the liposomal bilayer structure as an active component.

In addition, at least one kind of the following components should be included in the liposomes: soybean lecithin, yolk lecithin, distearoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, poloxamer, dimyristoyl phosphatidylcholine, tween, span, nonionic surfactant Brij, bile salt, cholesterol.

In the liposomes prescription liposome compositions and formulation of this the present invention, asiaticoside and lipid components of the liposomes account for 0.1~10% and 0.1~40% respectively.

The said organic solvents used according to the present invention include dichlormethane, chloroform, aether, ether, and ethanol.

The said aqueous solutions used according to the present invention include distilled water, deionized water, purified water, and phosphate buffer.

A method for the preparation of a liposomal emulsions containing ceramide is mentioned in CN 98110614.5. Whererin CN 98110614.5 in which drugs carried by the liposome is liposomes are provided with stable chemical property, which properties so that they are difficult to oxidize be oxidated, and have the function of skin protection such as damp-keeping, moisturizing, preventing drying and drying, desquamating, etc. These drugs can be easily absorbed by the skin. Therefore, the liposomes are perfect suitable as cosmetic additive additives and drug-carrier drug-carriers for

external use. The analogous Analogous methods in which liposomes are applied to the preparation of pharmaceutical preparations and ~~eosmetic cosmetics were are~~ disclosed by in ZL 96116044.6, CN 96192625.2, and CN 93114073.0.

Asiaticoside-liposome The asiaticoside-liposomes of this the present invention can be applied to the preparation of pharmaceutical preparations compositions and formulations and ~~eosmetic cosmetics~~. The asiaticoside-liposomes can ~~It could~~ be prepared by using conventional methods or the methods described in aforementioned patent documents. To form asiaticoside-liposome is useful to enhance Forming the asiaticoside-liposomes according to the present invention enhances the stability, skin penetrability and hydrophilicity of asiaticoside so that it is more convenient and logical suitable to prepare cosmetic or pharmaceutical preparations compositions and formulations containing the asiaticoside.

Asiaticoside-liposome The asiaticoside-liposomes of this the present invention are is primarily provided with the advantages as undermentioned: following advantages:

1. To enhance asiaticoside's The asiaticoside has enhanced stability. Drugs are enwrapped in the middle of liposomal bilayer, bilayers which can prevent the drugs from being destructed by instable factors such as light, oxygen, acid, base and so ~~on, consequently, drugs' on.~~ As a consequence, the stability of the drugs is enhanced. Liposomes It has been determined that the liposomes can enhance drug's the stability of drugs in both not only in vitro but and in vivo applications and also in vivo, it can prolong drug's action time of drugs in vivo. in in vivo applications.

2. To enhance asiaticoside's The asiaticoside has enhanced skin penetrability. Liposome Liposomes are drug carriers that are composed of lipid bilayer, bilayers which has have more comparability and compatibility with biological tissue, and can enhance drug's skin penetrability. penetrability of drugs. Liposome can Liposomes not only enhance drug's skin penetrability, penetrability of drugs, but also remain more retain larger quantity of drugs between

~~epidermis and dermis, however~~ dermis however, the dosage entering into the hematological system is decreased, so that general adverse effects can be efficiently avoided. ~~avoided efficiently~~.

Liposomes can enhance ~~drugs'~~ the skin penetrability of drugs by the mechanism of ~~hydration, fusion and penetration~~ fusion, penetration, etc. Furthermore, plentiful ceramides are contained in stratum corneum of human skin. According to similarity-compatibility theory, liposomes containing ceramides in lipid ~~bilayer~~ bilayers can further enhance ~~drugs'~~ skin penetrability and ~~absorbability~~ absorbability or drugs. ~~Asiaticoside-liposome~~ The asiaticoside-liposomes of this the present invention contain ceramides in the lipid ~~bilayer~~, they can bilayers which allows them to further enhance asiaticoside's the skin penetrability. penetrability of asiaticoside.

3. The asiaticoside-liposomes of the present invention can ~~be~~ mixed discretionarily with other components used in the prescription and compositions and formulations which make it more simple and convenient to prepare pharmaceutical preparations compositions and formulations and cosmetic cosmetics containing asiaticoside. In prescriptions compositions and formulations of most cosmetic cosmetics the ground substance is hydrophilic or ~~emulsive, thus emulsive~~. Thus, components of prescriptions the compositions and formulations should be hydrophilic or lipophilic. It is difficult to prepare cosmetic cosmetics containing asiaticoside because ~~of asiaticoside's~~ asiaticoside has bad hydrophilicity and lipophilicity. ~~Liposome is~~ Liposomes are a kind of drug carrier with high hydrophilicity, by which asiaticoside is encapsulated and the ~~drug's~~ hydrophilicity of the drug is thereby enhanced. ~~enhanced obviously, the~~ The encapsulated drug can then be mixed discretionarily with other components of the prescription compositions and formulations. It is more simple and convenient to prepare pharmaceutical preparations compositions and formulations and cosmetic cosmetics containing asiaticoside.

Detailed examples

Example 1:

30g asiaticoside, 20g soybean lecithin, 30g cholesterol, 40g poloxamer F₆₈, 10g ceramide, 200 ml chloroform, 100ml ethanol and 1000ml phosphate buffer (pH 7.4) were prepared.

Asiaticoside, soybean lecithin, cholesterol, poloxamer F₆₈ and ceramide aforementioned were placed into a 1000ml round bottom flask, and dissolved by the mixed in a solution of chloroform and ethanol, treated with the rotary ethanol. The resulting mixture was subject to a rotary thin layer evaporation technique in a thermostatic waterbath at a temperature of 25~40°C, and then so that a lipid film was afforded formed at the bottom of the flask. Then, 800ml phosphate buffer(pH 7.4) buffer (pH 7.4) was added to the flask, after flask. After the lipid film was hydrated under shaking, phosphate buffer(pH 7.4) buffer (pH 7.4) was added to the mixed solution to produce a volume of 1000ml, then 1000 ml. Thereafter asiaticoside-liposome was afforded produced after sonification (output4, (output 4, duty cycle 50%, time 20 mins).

Example 2:

50g asiaticoside, 50g yolk lecithin, 50g cholesterol, 20g ceramide and 1000ml phosphate buffer (pH 7.4) were prepared. Asiaticoside, yolk lecithin, cholesterol, and ceramide aforementioned were placed into a conical flask and fused by heating or dissolved in organic solvent stated in this invention to make lipid solution, then to produce a lipid solution that was placed in a thermostatic waterbath at a temperature of 80°C. 800ml phosphate buffer (pH 7.4) was placed in a waterbath till its temperature is was the same as the temperature of the lipid solution. Then an solution's, then aqueous solution and the lipid solution were mixed together while under shaking the mixture which was and then cooled. Phosphate cooled; phosphate buffer (pH 7.4) was added to the mixed solution to produce a volume of 1000 ml. 1000ml, after After homogenizing for 6 times with using a high pressure homogenization technique (higher pressure: 60MPa, lower pressure: 10MPa), asiaticoside-liposome was afforded produced.

Example 3:

20g asiaticoside, 20g dipalmitoyl phosphatidylcholine, 30g poly-dioxyvinylcetylether, 40g cholesterol, 40g ceramide, 200ml dichlormethane, 200ml ethanol and 1000ml phosphate buffer (pH 7.4) were prepared. ~~Asiaticoside, dipalmitoyl phosphatidylcholine, polydioxyvinylcetylether, cholesterol and ceramide aforementioned were placed into a 1000ml round bottom flask, and dissolved in the a mixed solution of dichlormethane and ethanol by heating, treated with the ratory heating.~~ The resulting mixture was subjected to a thin layer evaporation technique in a thermostatic waterbath at a temperature of 25~40°C, and then to produce a lipid film was afforded at the bottom of the flask. Then, 800ml phosphate buffer (pH 7.4) was added to the flask, after flask. After the lipid film was hydrated under shaking, phosphate buffer (pH 7.4) was added to the mixed solution to produce a volume of 1000 ml. 1000ml. The mixed solution was filtrated extrudedly from poly-(carbonic acid fibrous tunic) and then asiaticoside-liposome was afforded. obtained.

Example 4:

Stability experiment

~~The three groups of Samples of each of the asiaticoside-liposome products produced in Examples 1-3 and an aforementioned and asiaticoside aqueous solution were placed airtight containers respectively at a temperature of 40°C, and a relative humidity 75%. The content of asiaticoside in asiaticoside-liposome samples and the asiaticoside aqueous solution was determined by HPLC after 0, 1, 2, 3 month. months. The content of asiaticoside in asiaticoside-liposome samples and the asiaticoside aqueous solution was assumed to be 100% at 0 month, the month. The content of asiaticoside at other time times was obtained comparing with it at 0 month, then the percentage that the content amount of drug changed with time was obtained. The result indicated that after placed for three months at a temperature of 40°C, and a relative humidity 75%, the content of asiaticoside in asiaticoside-liposome samples changed a little, but the content of asiaticoside in the~~

asiaticoside aqueous solution had decreased. It proved This proves that asiaticoside encapsulated by liposomes could enhance ~~drug's stability obviously~~ drug stability.

Table 1 was the comparison of asiatoside's stability in liposomes and aqueous solution.

Table 1.

The variety percentage of asiacoside's content (%)				
Time (month)	0	1	2	3
Liposomes	100.00	87.56	75.41	68.02
Aqueous solution	100.00	99.52	98.69	98.12

n=3